UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Ex parte MARSCHALL S. RUNGE,
BENNETT VAN HOUTEN, and SCOTT W. BALLINGER

Appeal 2007-0582
Application 09/832,069
Technology Center 1600

Decided: April 23, 2007

Before DONALD E. ADAMS, ERIC GRIMES, and
RICHARD M. LEBOVITZ, *Administrative Patent Judges*.

GRIMES, *Administrative Patent Judge.*

DECISION ON APPEAL

This is an appeal under 35 U.S.C. § 134 involving claims to a method of predicting atherosclerotic heart disease based on mitochondrial DNA damage. The Examiner has rejected the claims as nonenabled and indefinite. We have jurisdiction under 35 U.S.C. § 6(b). We reverse.

BACKGROUND

The Specification states that "there is growing evidence that atherosclerotic lesions result from factors mediated by reactive oxygen

species" (Specification 4: 1-2). "Numerous studies have implicated the mitochondria as a vulnerable target for reactive oxygen species" (*id.* at 5: 1-2).

The Specification states that because "mitochondrial DNA is more susceptible to reactive oxygen species-mediated damage, and because increased oxidative stress is believed to play a role in the early events of atherogenesis, . . . the mitochondrial DNA in aortic tissues destined to become atherosclerotic has increased damage" (*id.* at 7: 1-6). The Specification discloses

> methods of predicting coronary atherosclerotic heart disease . . . based upon the extent of mitochondrial DNA damage or upon related measurement of mitochondrial dysfunction that is the result of mitochondrial DNA damage including changes in mitochondrial protein production, changes in mitochondrial oxidative phosphorylation or changes in mitochondrial ATP production.

(*Id.* at 8: 20 to 9: 5.)

DISCUSSION

1. CLAIMS

Claims 6, 8, 9, and 14-23 are pending and on appeal. Claims 6 and 16 are representative and read as follows:

> 6. A method of measuring the amount of oxidative stress in a human individual, comprising the steps of:
>
> (a) collecting a blood sample from said individual;
>
> (b) assessing the amount of mitochondrial DNA damage in cells from said sample wherein such amount of damage is indicative of oxidative stress in said individual.

16. The method of claim 6, wherein said mitochondrial DNA damage is assessed by measuring mitochondrial mRNA production.

2. PRIOR ART

The Examiner relies on the following references:

Corral-Debrinski et al., "Association of mitochondrial DNA damage with aging and coronary atherosclerotic heart damage," *Mutation Research*, Vol. 275, pp. 169-180 (1992).

Lenaz, "Role of mitochondria in oxidative stress and ageing," *Biochimica Biophysica Acta*, Vol. 1366, pp. 53-67 (1998).

Williams et al., "Increased oxidative damage is correlated to altered mitochondrial function in heterozygous manganese superoxide dismutase knockout mice," *Journal of Biological Chemistry*, Vol. 273, pp. 28510-28515 (1998).

Hudson et al., "Age-associated change in mitochondrial DNA damage," *Free Rad. Res.*, Vol. 29, pp. 573-579 (1998).

3. INDEFINITENESS

Claims 6, 8, 9, and 14-23 stand rejected under 35 U.S.C. § 112, second paragraph, as indefinite

> because it is unclear whether the final clause of the method is directed to detecting amount of damage or mere presence of damage. The claim states, "wherein such damage is indicative of oxidative stress in said individual." Thus, the claim does not particularly appear to require establishing a correlation based upon any amount or ratio or other measurement of quantity of damage.

(Answer 8.)

We will reverse this rejection. The Answer does not accurately quote the claim language. Claim 6 states the "amount of damage is indicative of

oxidative stress." The claim language makes clear that oxidative stress is determined based on the *amount* of mitochondrial DNA damage, not the mere presence of damage.

4. ENABLEMENT

Claims 6 and 16-20 stand rejected under 35 U.S.C. § 112, first paragraph, on the basis that the specification,

> while being enabling for a method for measuring the amount of oxidative stress in an individual by detecting the amount of DNA damage per length of DNA using QPCR, does not reasonably provide enablement for detecting mtDNA damage by measuring mitochondrial mRNA production, mitochondrial protein production, mitochondrial oxidative phosphorylation, mitochondrial ATP production or mitochondrial redox state.

(Answer 3.)

Thus, the Examiner does not dispute that atherosclerotic heart disease can be predicted by measuring mitochondrial DNA (mtDNA) damage; the issue is whether mtDNA damage can be measured indirectly. Appellants identify this as the real issue in the case (Br. 5-6) and the Examiner agrees (Answer 11: "'whether the assay is reasonably predictive of mtDNA damage' . . . [is] the true question of enablement in the instant application").

The Examiner argues that the

> specification provides no evidence teachings regarding the relationship between mtDNA damage and mitochondrial mRNA production, mitochondrial protein production, mitochondrial oxidative phosphorylation, mitochondrial ATP production or mitochondrial redox state. The specification does not teach how these measurements are associated.

(Answer 5.) The Examiner also states that the "specification has no working examples of measuring the *amount* of mtDNA damage in tissue using mitochondrial mRNA production," etc. (*id.* at 6).

Appellants disagree with the "Examiner's position that there is no exemplary support in the specification" (Br. 8). Appellants point to Examples 5-7 in the Specification, which they characterize as showing that oxidative damage causes changes in mitochondrial mRNA production, protein production, oxidative phosphorylation, ATP production, and redox state (*id.*). Appellants argue that the Examiner's reasoning in support of the rejection is based on the effects of single mutations, while the claimed method measures the cumulative effect of mutations in a population of mitochondria (*id.* at 9).

We agree with Appellants that the Examiner has not provided an adequate basis for concluding that the measurements recited in claims 16-20 would not be reasonably predictive of the amount of mitochondrial DNA damage.

The Specification provides several relevant working examples, describing experiments in which human umbilical vein endothelial cells (HUVEC) and human aortic smooth muscle cells (HASMC) were treated *in vitro* with the reactive oxygen species hydrogen peroxide (H_2O_2) and peroxynitrite ($ONOO^-$) (Specification 27-39).

The Specification reports that "[h]ydrogen peroxide treatment resulted in increased mitochondrial DNA damage in both cell lines" (*id.* at 41: 6-7), and "[p]eroxynitrite treatment resulted in preferential damage to the mitochondrial DNA [compared to nuclear DNA] in HUVEC and HASMC"

(*id.* at 42: 9-10). The damage was reported to be significant in both cell types, treated with either reactive oxygen species (*id.* at 64: 22-29).

The Specification also reports that:

• "Treatment of HUVEC and HASMC with peroxynitrite resulted in substantially decreased transcript levels in the mitochondrial encoded genes, NADH dehydrogenase 2 (ND2) and cytochrome b (Cyt b), but not the 16S rRNA" (*id.* at 43: 20 to 44: 2);

• "Reactive oxygen species treatment also resulted in a decrease in mitochondrial protein synthesis in both cell lines" (*id.* at 45: 13-14), with decreases of 12% to 70% depending on the concentration of reactive oxygen species (*id.* at 46: 10-15); and

• "Peroxynitrite treatment also resulted in an overall decrease in ATP levels and mitochondrial respiration (complex II) in HUVEC and HASMC" (*id.* at 46: 17 to 47: 1), with "significant decreases in ATP" and "a significant decrease of complex II reduction of MTT (mitochondrial respiration)" (*id.* at 47: 4-7).

Thus, the Examiner is correct in stating that the Specification does not provide "working examples of measuring the *amount* of mtDNA damage in tissue using mitochondrial mRNA production," etc. (Answer 6); i.e., the examples do not provide formulas to convert a specific amount of, e.g., mitochondrial mRNA production to a specific amount of mtDNA damage.

However, the Specification provides convincing evidence of a *correlation* between increased mitochondrial DNA damage and decreased mitochondrial mRNA production, protein production, ATP levels, and respiration. The claims do not require precise, quantitative determination of

the amount of mitochondrial DNA damage: the Specification states that increased mitochondrial DNA damage indicates increased oxidative stress. The Examiner has not adequately explained why the correlations shown in the working examples do not allow those skilled in the art to the make type of qualitative determination (normal range vs. abnormal) that is required to practice the claimed method.

The Examiner also argues that the effect of a given mutation is unpredictable: depending on its type and location, a mutation can completely abolish the function of a gene product or it can have no effect at all (Answer 5). Because a given mutation can have a range of effects on mRNA production, protein production, etc., the Examiner concludes that measuring such "downstream" effects cannot be relied on to measure the amount of DNA damage (*id.* at 5-6).

We agree with Appellants that the Examiner's concern is misplaced. As Appellants have pointed out,

> [w]here there is a *population* of mitochondria being tested (which there certainly would be if one were testing a blood sample or blood products as stated by the claim), there would necessarily be a vast range of mutations occurring in the mitochondria, some within coding regions and some outside of a particular coding region. Nevertheless, the distribution would be expected to be random and thus demonstrate a readily identifiable *correlation* between the amount of damage in any one gene, as measured by expression of that gene, and the amount of damage overall.

(Br. 9.) We agree with Appellants' analysis, and note that it is supported by the correlations demonstrated in the Specification's working examples.

SUMMARY

The Examiner has not established that the claims are indefinite or that undue experimentation would have been required to practice the claimed method. We reverse the rejections under 35 U.S.C. § 112, first and second paragraphs.

REVERSED

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